London County Council.

REPORT

ON THE

RESULT OF INVESTIGATIONS

ON THE

MICRO-ORGANISMS OF SEWAGE.

PART I.

Their relation to those of Sewer Air.

PART II.

Observations on the Bacillus of Typhoid Fever and its relation to Sewage.

BY

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(*Printed by order of the Main Drainage Committee, 13th December*, 1894.)

No. 216. *Price* 4d. *Sold by Edward Stanford, 26 and 27, Cockspur-street, Charing-cross, S.W.*

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REPORT

ON THE

RESULT OF INVESTIGATIONS ON THE MICRO-ORGANISMS OF SEWAGE.

To the Main Drainage Committee of the London County Council.

In previous reports made to the London County Council by one of us, it was shown that the micro-organisms contained in sewer air were not only less in number than the micro-organisms in fresh air in the vicinity at the same time, but that they were also apparently related to and derived from those of fresh air rather than from the sewage, and further that no evidence was forthcoming that sewage was able to give off micro-organisms to the air in contact with it. Inasmuch as these conclusions are not in harmony with generally accepted views, it became of great importance to gain corroborative evidence upon this point by a study of the organisms existing in sewage, and to this end we were instructed by your committee to investigate the matter. This was the more essential because practically no exact study of the subject had been previously made, at least in this country. By far the most important work on the micro-organisms of sewage which has yet been done is that of Mr. E. O. Jordan, published in the Reports of the State Board of Health of Massachusetts (1888–90, Part II.). Mr. Jordan reports on and carefully describes twelve species of bacilli, most of them previously unknown, isolated at the experimental sewage station at Lawrence, Mass., and further adds an important report on nitrification and the organism concerned in the process. In the main the results which we have attained are in accord with those of Mr. Jordan, and such differences as we have found may be explained by the probable differences which exist between the floras of English and American waters, and the sewages of London and Lawrence. The work of Roscoe and Lund* does not cover the same ground as that which we have attempted to explore. Their work deals more especially with the chemical properties of a few species of bacteria, chiefly bacilli, isolated from Acton sewage.

PART I.

THE MICRO-ORGANISMS OF SEWAGE.

Methods adopted.—1. A mode of collection of the sewage was adopted which should give a fair average sample. It is obvious that, especially in small sewers, the rapid variation in the composition of the sewage may render a given amount taken at a particular moment a very unfair sample of the whole bulk. We therefore took repeated small quantities during a period of fifteen to twenty minutes, thereby gaining a fair sample of the sewage in question. These were mixed in a large sterilised bottle (a "Winchester quart"), and subsequently the whole was well incorporated by vigorous shaking for twenty or thirty minutes, in order to break down as far as possible any solid matter. In every case cultivations were made as soon as possible after collection, since, if three or four hours are allowed to elapse, especially in warm weather, the rapid multiplication of the micro-organisms present necessarily vitiates the results.

2. The mode of dilution employed, which was designed to secure an equable admixture of the sewage with the large amount of diluent required, was as follows—Ten cubic centimetres of the mixed and shaken sewage were diluted to 100 c.c. with recently boiled distilled water in a sterilised and accurately stoppered flask, and thoroughly shaken. Of this first dilution 10 c.c. were taken and further diluted to 1,000 c.c. with sterilized distilled water in a second sterile flask and again well shaken. From this second dilution (which represents 1 in 1,000) two further dilutions were made in similar manner—one by taking 10 c.c. and diluting to 100 c.c. (giving a dilution of 1 in 10,000), and another by taking 10 c.c. and diluting to 1,000 c.c. (giving a dilution of 1 in 100,000). These extreme degrees of dilution were of course rendered necessary by the enormous numbers of micro-organisms present in even fresh sewage. A subsequent experiment indicated the possibility that a certain number of bacteria may have been killed off by the distilled water. A better diluent would probably have been sewage sterilised by filtration through a Chamberland or Berkefeld filter. The possible fallacy thus introduced will, however, only be that our results in point of numbers may be somewhat too low. The reason for employing two dilutions (of 1 in 10,000 and 1 in 100,000 respectively) was that our plate cultures were incubated at two different temperatures, some at the temperature of the body (37°C.), and some at a temperature of 22°C., and, inasmuch as many sewage bacteria do not grow at the higher temperature, a lesser degree of dilution could be employed.

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* Transactions of the Royal Society, 1892.

3. The chief mode of cultivation employed was that of agar-agar plates, mainly because a large number of the bacteria of sewage rapidly liquefy gelatine, and also because in certain cases the plates were incubated at 37° C. By the ordinary device of keeping the plates upside down all trouble from the condensation of water was avoided. It is true that gelatine is in general a better medium than agar-agar for the growth of micro-organisms, and subsequent experiments indicated that we should possibly have found larger numbers had we used it, nevertheless the presence of liquefying organisms made its employment almost impossible. Before the more slowly growing colonies have time for development the plates become useless from the rapid liquefaction of the gelatine. Our attention was also specially directed to the possibility of the occurrence of certain disease germs in the sewage, notably those of typhoid, cholera, and diphtheria, and these three organisms all grow well on agar-agar.

From each sample of sewage taken, 6 to 10 plates were made, and the number of colonies which appeared showed an agreement which convinced us that the method of dilution adopted was a good one.

4. *Methods of examination.*—Each plate was examined from day to day, and the number of colonies counted accurately with a hand lens. When the number became constant, *i.e.*, when new colonies had ceased to appear, an average was struck between the different plates. Calculation of the total number of micro-organisms was made from the plates which had been incubated at 22° C., since very many bacteria do not grow at a temperature of 37° C. In our latter experiments greater accuracy was obtained by making careful drawings of each plate and numbering the different colonies.

From every kind of colony which appeared, so far as we were able to judge, sub-cultures were made, and these were identified and named as completely as the means at our command allowed. This involved not only microscopic observations, but sub-cultures in all the ordinary media employed, agar-agar, gelatine, beef-broth, milk, potatoes, &c., and also in many cases the use of chemical tests. But in a very large number of cases, owing to imperfect descriptions and to the fact that many of the bacteria with which we met seem to be hitherto undescribed, identification has been impossible. In a few cases we give descriptions of such new species as appear to us of importance, but in most instances we are obliged to pass over new or undescribed species, as to work out all their characters would be the labour of some years.

Inasmuch as we desired to keep a very careful watch for the occurrence of the typhoid fever bacillus in sewage, and since this organism is very similar to Bacillus coli communis (a normal inhabitant of the human intestine and one of the commonest of sewage bacteria), and indeed only to be distinguished from it with certainty by various chemical reactions, it was necessary to employ special chemical tests in the case of all colonies which seemed likely to be the typhoid bacillus. Particulars of the methods employed will be given later, when we describe in detail our search for this organism in sewage.

It was clearly probable that the bacteria present in fresh sewage might prove very different from those in older and more decomposed sewage. Sewage may be a favourable medium for the growth of one species, an unfavourable one for another. The latter will therefore tend to disappear from older sewage, which will contain larger and larger numbers of those particular organisms for which it is a suitable soil. We therefore determined to examine, in the first instance, the most recent and unaltered sewage to which we could gain access, but which nevertheless should be a fairly representative sample. We selected the Smithfield outfall from St. Bartholomew's Hospital, as the area drained by this sewer is sufficiently large to give a fair sample of average fresh sewage, including as it does, not merely that from hospital wards but from several dwelling houses, and laboratories of various kinds. The hospital has, moreover, during the past few years been newly drained, and the sewage is delivered at the outfall within a few minutes of its discharge into the drains, having no prolonged sojourn in old and foul brick drains to alter its composition. We hoped thus to gain some idea of the bacterial flora of perfectly recent and unaltered sewage.

We next examined a sample of somewhat older sewage from a much larger area, but including that from St. Bartholomew's Hospital—that, namely, from the Fleet sewer in Farringdon-street under Holborn Viaduct, which takes the sewage from the districts of Holborn and Smithfield. Later we examined sewage from the Barking outfall, which represents the sum total of all sewage north of the Thames, and from the Crossness outfall, which is a similar summation of all sewages south of the Thames. Ultimately, with the special object of searching for the bacillus of typhoid fever, we examined sewage from the drain which leaves the scarlet and typhoid fever blocks at the Eastern Fever Hospital at Homerton, and again that from a sewer in Bridge-street, Homerton, about a quarter of a mile from the hospital, which conveyed the sewage of the Homerton district, including the hospital, into the main sewer.

THE TOTAL NUMBER OF BACTERIA PRESENT IN SEWAGE.

Various published determinations show the number of organisms present in sewage to be enormous. The results of Mr. Jordan's examination of the sewage of Lawrence, Mass., gave an average of only 708,000 living bacteria per cubic centimetre, his highest result being 3,963,000 per c.c. He obtained far higher results during the summer months than at other times. We have found, and our results agree with those of other observers in England, that the number of bacteria in London sewage is considerably higher than this. Determinations of the number of micro-organisms in the sewage of the King's Scholars' Pond sewer in November and December, 1891, mentioned in a previous report, showed 2,618,000 and 3,179,000 respectively. The figures we have obtained are as follows:—

In perfectly fresh sewage from St. Bartholomew's Hospital on January 26th, 1894, at 10.30 a.m. from which cultivations were made immediately, without allowing any time for the multiplication of the micro-organisms, we found on an average 2,781,650 bacteria per cubic centimetre. Two agar-agar plates, inoculated each with 1 c.c. of the sewage diluted 10,000 times, and incubated at 22° C. yielded

respectively 216 and 330 colonies. Six similar plates, inoculated each with 1 c.c. of the sewage diluted 100,000 times, yielded respectively 21, 39, 26, 27, 28, and 29 colonies. The total average is given above. The same sewage, however, after being kept for three days in a stoppered bottle, showed an enormous increase in bacteria, a similar plate inoculated with 1 c.c. of the sewage diluted 100,000 times yielded 338 colonies, representing 33,800,000 bacteria per cubic centimetre.

From the Fleet sewer in Snow-hill the results were somewhat higher. The sewage was taken on March 2nd, at 11.30 a.m., and cultivations were made immediately. Three agar-agar plates, made in the same way as the above, with 1 c.c. each of the sewage diluted 100,000 times, gave respectively 40, 28, and 34 colonies, yielding an average of 3,400,000 bacteria per cubic centimetre. With this sewage also we made further experiments, to ascertain what proportion of the bacteria present would grow at a temperature of 37° C., or with the addition of ·05 per cent. carbolic acid, or under the influence of both these conditions combined. Two agar-agar plates, inoculated each with a cubic centimetre of the sewage diluted 10,000 times, with the addition of ·05 per cent. carbolic acid, and incubated at 37° C. yielded respectively 59 and 38 colonies, giving an average of 485,000 organisms per cubic centimetre which could grow under these unfavourable conditions. Two plates made in a similar manner, without the addition of carbolic acid, but incubated at 37° C., yielded respectively 103, and 78 colonies, or an average of 905,000 colonies per c.c., which could grow at this temperature. A single plate inoculated with 1 c.c. of a dilution of 1 in 100,000, with the addition of ·05 per cent. carbolic acid, but incubated at 22° C., yielded 19 colonies, or 1,900,000 organisms per c.c. of sewage. These results indicate that a temperature of 37° C. inhibited the growth of 73·4 per cent. of the total number of organisms present in this sewage, while the addition of ·05 per cent. carbolic acid inhibited the growth of 44·2 per cent. of the total number; the combination of ·05 per cent. carbolic acid, with a temperature of 37° C., inhibited the growth of no less than 85·8 per cent. of the bacteria present.

In the case of the sewage collected at Barking, at 11.30 a.m., on April 4th, an hour and a-half elapsed before cultivations could be made in the laboratory. Two agar-agar plates, made each with 1 c.c. of the sewage diluted 100,000 times, and incubated at 22° C., yielded respectively 21 and 17 colonies, or an average of 1,900,000 bacteria per c.c. of sewage. A gelatine plate, however, made in the same way gave 64 colonies, or 6,400,000 bacteria per c.c. of sewage. It is known that gelatine is a more favourable medium for the growth of bacteria than agar-agar, but the discrepancy between these two results is surprising; it is easy, however, to attach too much importance to isolated observations of this kind. The number of bacteria calculated from the agar-agar plates is less than might have been expected, but the results given by similar plates kept at a higher temperature, or with the addition of ·05 per cent. carbolic acid, are proportionate. With the same sewage at the same time, two similar agar-agar plates (each containing 1 c.c. of the sewage diluted 10,000 times) were inoculated and incubated at 37°C.; one yielded 27 and the other 60 colonies, or an average of 435,000 bacteria per c.c. of sewage, able to grow at the higher temperature: *i.e.*, 77·2 per cent. were inhibited in their growth. Two agar-agar plates containing each 1 c.c. of the sewage diluted 100,000 times, but with the addition of ·05 per cent. carbolic acid, yielded when incubated at 22° C., 11 and 8 colonies respectively, equal to 950,000 organisms per c.c. of sewage able to grow in the presence of this amount of carbolic acid: *i.e.*, 50 per cent. were inhibited in their growth. Lastly, the two conditions were combined and two agar-agar plates were inoculated with 1 c.c. each of the sewage diluted 10,000 times, and incubated at 37° C. with the addition of ·05 per cent. carbolic acid; these yielded respectively 15 and 8 colonies, or an average of 115,000 organisms per c.c. of sewage able to grow at 37° C. with the addition of the above percentage of carbolic acid: *i.e.*, 94 per cent. were inhibited.

With regard to the total number of organisms present in sewage the highest results were obtained from a sample taken at the Crossness outfall at 2.30 p.m. on July 10th. This is doubtless attributable on the one hand to the warmer weather then prevailing, and on the other to the fact that three hours elapsed between the time the sewage was collected and the making of the cultivations. The possible effects of this interval were however obviated as far as possible by keeping the bottle of sewage during its transit packed in a chloride of ammonium freezing mixture. Six agar-agar plates were made, each containing 1 c.c. of the sewage diluted 100,000 times, and they were incubated at 22° C. They yielded respectively 124, 127, 105, 119, 110 and 88 colonies, or an average of 11, 216, 666 micro-organisms per c.c. of sewage.

The sewage collected from the fever hospital at Homerton on May 23rd at 11 a.m. was examined chiefly for the purpose of discovering the typhoid fever bacillus. Of two agar-agar plates made with 1 c.c. of the sewage diluted 100,000 times, and incubated at 22° C, one gave 45 and the other 35 colonies, or an average of 4,000,000 bacteria per c.c. of sewage. Two gelatine plates made at the same time and of the same degree of dilution yielded after two days 41 and 42 colonies, or 4,150,000 bacteria per c.c. of which 2,600,000 liquefied gelatine. But many more colonies would have appeared but for the rapid liquefaction of the gelatine, so that this is not to be regarded as a result fairly comparable with that of the agar-agar plates.

A similar experiment with agar-agar plates, made with sewage taken from the Bridge-street sewer at Homerton on June 21st, at 2.30 p.m., gave an average of 4,050,000 bacteria per c.c. of sewage.

It will thus be seen that very wide variations exist in the total number of micro-organisms present in sewage at different times and in different places, as indeed might safely have been predicted. Temperature is one important factor in determining the rapidity of their reproduction, and hence the increase in their numbers; dilution of the sewage by rainfall must also exert a marked influence. The estimations on which the foregoing figures are based were made with all possible care, and we believe them to be as accurate as possible for each particular sewage at the time we examined it. But it is clear that a small number of determinations, such as we have made, can afford only an indication of the average number of bacteria present in sewage, nor was it our desire to pursue this subject in detail, as we were concerned with the nature of the organisms present rather than with their actual numbers.

SPECIES OF MICRO-ORGANISMS PRESENT IN SEWAGE.

1.—*Fresh sewage from St. Bartholomew's Hospital.*

From the plates made from this sewage more than 200 colonies were examined, sub-cultures in various media being made from most of them. The following organisms were recognised—

Moulds.—One colony alone was found, of a dark brown colour, and identical with the species found in sewer air.

Yeasts.—Four colonies of *torula* grew, belonging to at least two species. One species was white and allied to common yeast (S. cerevisiæ) the other was of a pale pink colour.

Micrococci.—A small *streptococcus* was the commonest organism present in this sewer, no less than 37 of the colonies were referred to this species. The colonies were minute and grew slowly, so that they formed a very inconspicuous feature in the plates. We were unable to identify the exact species, but it possessed the power of coagulating milk in 24 to 36 hours when incubated at 37° C. It was clearly not the common pathogenic species (Streptococcus pyogenes).

Micrococcus ochroleucus occurred twice.

Micrococcus luteus twice.

Pediococcus albus doubtfully once.

A citron coloured micrococcus which we failed to identify was met with three times.

A new species of pink coccus, which we have named *Micrococcus aurora*, and which we describe later, occurred once.

Sarcina colonies were found eight times, all being of a yellow colour, though none could be referred to the common sewer air and fresh air sarcina (S. lutea). Amongst them were *S. flava* and *S. aurantiaca* and at least two other species which are apparently undescribed.

Bacilli.—No less than 45 colonies resembled at first sight *B. coli communis*, but of these less than a dozen responded to the chemical tests (coagulation of milk, formation of gas bubbles in gelatine shake-cultures, and production of indol in broth) which are relied on to distinguish the bacillus from its allies. It was, nevertheless, if we except the above-mentioned streptococcus, the commonest and certainly the most conspicuous of the organisms present in this sewage. Those of the 45 colonies which failed to give some or all of the three chemical tests must be classed, from their morphological and cultural resemblances, as close allies of B. coli communis, but we are unable to refer them to any described species.

B. fluorescens liquefaciens was met with twice.

B. aureus occurred three times.

B. janthinus, a brilliant violet species, occurred in one colony only.

A dark orange brown bacillus, growing rapidly, was found once.

B. subflavus and *B. fluorescens aureus* were each met with doubtfully once.

Proteus Zenkeri, a common putrefactive organism, occurred twice.

Bacillus mycoides occurred twice.

A bacillus allied to *B. pyocyaneus*, which we describe later as *Bacillus cloacae fluorescens*, occurred twice.

A very rapidly growing bacillus, which we describe later as *Proteus cloacinus*, was met with once. This species was present in all the different samples of sewage which we examined.

Cladothrix.—Cladothrix dichotoma was found once.

2.—*Snow-hill sewage (Fleet sewer).*

In all we examined 365 colonies in the plates made from this sewage. The variety of species was somewhat less than in the fresh sewage from St. Bartholomew's Hospital: we recognised 15 species as compared with 22 from the latter.

Moulds.—One colony of brown mould only grew in the plates from this sewage.

Torula—A white torula occurred several times.

Micrococci.

The *streptococcus* coagulating milk, mentioned as common in the St. Bartholomew's sewage, was again very abundant here; 22 colonies were isolated.

Staphylococcus cereus albus occurred twice.

Staphylococcus pyogenes citreus was present four times.

Staphylococcus pyogenes aureus occurred once.

Diplococcus albicans tardissimus, eight colonies were found.

Diplococcus roseus—This organism, present also in sewer air, occurred once, and was again met with in other sewage.

Micrococcus flavus liquefaciens—One colony only met with.

Sarcina flava occurred in 25 colonies together with three colonies of an apparently new species of *yellow sarcina*, giving a characteristic sepia brown reaction with strong sulphuric acid.

Cladothrix, C. dichotoma, two colonies.

Bacilli.—Here again *B. coli communis* was one of the commonest bacilli present, but of the numerous colonies which at first sight resembled it only a small proportion, a dozen or so in all, gave all the typical chemical reactions. But it is worth noting that some of these after six to eight weeks in sub-culture, while retaining the power of coagulating milk, lost the power of producing indol in broth, and in some cases of producing gas bubbles in gelatine shake cultures. There were present a large number of colonies of species allied to B. coli communis, which we could not identify with any already described organism, some of these gave two of the chemical reactions of B. coli communis, but not the third.

B. mesentericus ruber was found once.

B. aureus occurred once.

Proteus cloacinus, described below, occurred twice.

A species resembling *B. aquatilis sulcatus* was found once.

One colony was found which at first sight resembled the diphtheria bacillus, but which on sub-culture proved not to be this species.

3.—*Barking sewage.*

In this experiment we examined 167 colonies. In this, the oldest sewage we were able to examine, we found a marked difference as to the number and character of the species of bacteria present compared with those of the more recent sewages.

Two moulds were found: one *penicillium glaucum*, one hitherto undescribed.

Four colonies of a pink *torula* were found which had the unusual property (in a torula) of very slowly liquefying gelatine. Also a white torula.

Streptococcus colonies were found five times.

A yellow *staphylococcus*, which we could not identify, was common, 27 colonies being found.

Diplococcus roseus occurred three times.

Sarcina flava was doubtfully present in one colony.

Bacillus aureus occurred only once.

B. coli communis was not found, and this fact constitutes one of the most striking features in this sample of sewage. Numerous colonies grew which resembled this species, but these when tested chemically, produced coagulation of milk in only 12 cases, and these 12 colonies failed to give the other chemical reactions of B. coli communis.

B. albus putidus, an organism which we had not previously met with, was, however, abundant in this sewage, 14 colonies being found. A similar prevalence of this species was found later in the Crossness sewage.

4.—*Crossness sewage.*

No careful study was made of the bacteria present in this sewage, owing to the fact that we were at this period chiefly devoting our attention to a search for the typhoid bacillus, and to its growth in sewage under varying conditions.

We may note, however, that out of 673 colonies which grew in the plates made from this sewage, only three were moulds; and indeed in the whole course of our observations on the bacteriology of sewage, only seven colonies of moulds occurred or an average of only 0·4 per cent. of the colonies examined.

The prevalence of *Bacillus albus putidus* was noteworthy, also the number of *Proteus cloacinus* described later.

5.—*Homerton sewage.*

Here our attention was almost exclusively devoted to a search for the typhoid fever bacillus, of which we succeeded in finding two colonies. We could pay little heed to other organisms present, though it may be noted that *B. coli communis* was present, and that we found enormous numbers of *Bacillus fluorescens stercoralis*, a species described below. *B. mycoides* was also found.

Of the organisms present in the sewage from Bridge-street, Homerton, we have no special notes, having been engaged solely in the endeavour to find the typhoid bacillus. Bacilli closely allied to B. coli communis were however present in considerable numbers.

DESCRIPTION OF CERTAIN NEW SPECIES OF BACTERIA IN SEWAGE.

So little has been done in this field that, had we had the time to devote to the subject, it would have been easy to describe many dozen species of bacteria which we have met with in the course of our observations, which have, so far as we can ascertain, not been hitherto described. The complete flora of so complex a fluid is however the work of a lifetime, and we must be content to describe a few forms only, which on account of their numerical prevalence, seem to us worthy of special notice, or which, in one case, are of special beauty.

The species we propose to name and describe are as follows:—

Micrococcus aurora.

Source.—Found in fresh sewage from St. Bartholomew's Hospital.

Form and arrangement.—Slightly oval cocci, 0·7 to 0·8 μ in diameter, often as diplococci separated by a cleft, rarely in short chains of 3 or 4 elements. In broth cultures the cocci are often arranged in irregular packets. Large irregular torula-like forms occurred once in an agar-agar culture.

Motility.—Non-motile.

Growth on gelatine plates.—Colonies first appear on the third day, minute and circular, not yet pink in colour. Under the microscope they appear granular and slightly irregular in outline. On the fifth day the surface colonies are less than 0·5 m.m. in diameter, circular, prominent, and pinkish. After a week the largest colonies are 0·7 m.m. in diameter, those in the depth 0·25 m.m., and of a pale pink tint. Later the colonies attain a diameter of 1 to 1·5 m.m. and liquefaction slowly occurs after two to three weeks.

Streak cultures on gelatine show after two days a narrow prominent line of growth of a pale pink tint, which eventually attains a width of 1·5 to 2 m.m. and a bright pink colour. Liquefaction occurs after 14 to 16 days.

Stab cultures in gelatine show definite growth in three days, almost entirely confined to the surface; in the depth of the gelatine little or no development occurs. A flat layer 4 m.m. in diameter and of a light pink colour is present on the surface at the end of a week. Liquefaction is extremely tardy, but some depression of the surface occurs after a fortnight, and distinct commencing liquefaction in 16 days.

On agar-agar.—*Streak cultures* at 20° C. show distinct growth in two days, semi-transparent and barely pink. At the end of a week the growth is opaque and of a bright pink colour, the streak being some 3 m.m. wide. At 37° C. no growth occurs.

On potato.—Grows exceedingly well, forming a thick layer of a brilliant deep pink colour, which eventually covers the whole surface.

In broth.—The fluid gradually becomes turbid, and after a week there is a light pink sediment at the bottom of the tube.

Temperature.—Grows well at 20° to 22° C. No growth on any medium at 37° C.

Rapidity of growth.—Grows rather slowly, but maximum of growth is attained in 7 to 10 days.

Spore formation.—Not observed.

Need of oxygen.—Growth very scanty away from the surface.

Gas production.—Not observed.

Action on gelatine.—Very slow and gradual liquefaction after two to three weeks.

Pigment production.—Produces a bright pink pigment, readily soluble in alcohol, only slightly so in water.

Relations.—Appears most closely related to *Micrococcus agilis* and *Micrococcus* roseus, but differs from the former in the absence of motility and the lighter pink colour of the cultures, and from the latter in the absence of any growth at 37° C.

Proteus cloacinus.

Source.—Found in every sample we have examined.

Form and arrangement.—A short bacillus of variable length: 0·4 to 0·5 μ in breadth; stained specimens from a two-days-old agar-agar culture vary from 0·5 to 1 μ in length; the shorter forms almost resembling cocci. On gelatine some longer forms are seen, up to 1·6 μ in length, and these may be as much as 0·6 μ in breadth. In a one-day-old broth culture the average length is 0·8 μ.

Motility.—Actively motile.

Growth on gelatine plates.—The colonies are visible to the naked eye in 19 hours, though still very minute. Under the microscope the surface colonies are thin, transparent and irregular in outline, the deeper colonies globular and brownish by transmitted light. In two days the surface colonies are more circular and regular in outline; the largest are about 1 m.m. in diameter; a few show a thin spreading edge, but no "swarming islands" are given off. No further change occurs except that the colonies increase in size up to 2 m.m. in diameter, and become raised at the edges; they are of a greyish white colour. No liquefaction occurs even at the end of a fortnight. The plates have a somewhat sour smell.

Gelatine streak cultures.—Growth is rapid—the streak attaining a width of 1·5 m.m. in 24 hours, when it is semi-transparent and whitish, with a gummy appearance. The edges are irregular, but show none of the characteristic "swarms" given off by *Proteus vulgaris*. In two days typical streaks are white and semi-opaque with a waxy lustre, and in five days the width of the streak may be 3 to 5 m.m. Later the growth becomes distinctly terraced, and liquefaction eventually occurs in about three weeks. The species is, however, very variable in its behaviour in gelatine streak culture. Specimens, the purity of which was assured by subsequent plate culture, occasionally liquefied the gelatine in from three to five days, and in these cases "swarming islands" were thrown out from the edges of the streak as in the common Proteus. The "swarms" are thrown out into the depth of the gelatine as well as laterally, and the process seems associated with early liquefaction. In other cases streaks grew with much lateral expansion, forming an irregular semi-transparent growth on the surface like that of B. coli communis.

Gelatine stab cultures.—Growth is rapid and well marked all along the needle track. Scanty gas bubbles are formed. Liquefaction first commences in from two to three weeks. The growth is white and nodular in the depth.

Gelatine shake cultures.—There is a profuse formation of gas bubbles in 24 hours; these are later partially re-absorbed.

Agar-agar.—*Agar-agar plates* incubated at 37° show exceedingly rapid and characteristic growth. A single colony spreads in 36 hours, as a thin irregular dendritic film covering almost the whole surface of the plate. The film does not thicken on further incubation.

Agar-agar streak cultures.—Growth is very rapid both at 37° C and 20° C. Even at the latter temperature the whole surface of the tube is covered in 24 hours by a thin homogeneous semi-translucent greyish film, advancing at the edge by irregular processes. On the second day the growth is somewhat thicker and whiter and has reached its full development. There is a scanty development of gas bubbles in the depth of the agar-agar, but no odour of putrefaction is ever perceptible.

Potato.—Grows rapidly, forming a slimy layer of a yellowish grey colour. The bacilli grow to longer threads than on other media, and become irregularly swollen.

Broth.—In 24 hours the broth is turbid, and in a few days a slight film is present on the surface, and a yellowish white deposit at the bottom of the tube. In a week there is a marked putrefactive odour (absent in gelatine and agar-agar cultures). Tested, after 11 days, with nitric acid—containing nitrous acid—no trace of indol reaction was obtained.

Milk.—Grows without producing any change in this medium.

Temperature.—Grows equally well at 37° C and 20° C.

Rapidity of growth.—Grows very rapidly, and on agar-agar with extraordinary quickness.

Spore formation.—Not observed.

Need of oxygen.—Grows well in the depth in stab cultures.

Gas production.—Abundant.

Action on gelatine.—Variable. Typically, no liquefaction occurs till after a lapse of two or three weeks; exceptionally, it occurs in three to five days.

Pigment production and chemical reactions.—Produces no pigment. Decomposes broth with the evolution of a strong putrefactive odour. No such change noted in other media. Does not give the indol reaction.

Relations.—Belongs apparently to the group of bacilli included formerly under the term '*Bacterium termo.*' Resembles in many respects *Proteus mirabilis*, but as a rule liquefies far more slowly and only occasionally throws out "swarming islands."

Bacillus cloacae fluorescens.

Source.—Found in Homerton sewage and sewage from St. Bartholomew's Hospital.

Form and arrangement.—Motile rods, on an average twice as long as broad, though some are longer. Breadth 0·6 to 0·75 μ, length 1 to 2 μ, the average being about 1·3 μ. The rods are sometimes attached in chains.

Motility.—Actively motile.

Growth on gelatine—Gelatine plates.—Minute colonies first become visible in 24 hours. In two days the surface colonies have begun to liquefy and are circular, 2 to 2½ m.m. in diameter, and under the microscope are seen to have a very delicate transparent irregular spreading margin not yet liquefied. The circular liquefied portion is turbid with a granular deposit in the centre, and shows slight but distinct greenish fluorescence. On the third day liquefaction has advanced so far that the colonies are 5 or 6 m.m. in diameter and partly fused with one another, the whole plate being largely liquefied with bright yellowish green fluorescence and a sour and unpleasant smell.

Gelatine stab cultures.—Growth occurs almost entirely at the surface, along the depth of the needle track hardly any is to be seen. Liquefaction begins at the surface on the second day, in a cone some 2 m.m. wide at the surface, extending down some way along the needle track. It gradually spreads, chiefly at the surface, till it reaches the walls of the tube in seven days, by which time slight fluorescence is visible. In a fortnight the gelatine is liquefied for 10 or 12 m.m. from the surface, and spreads downwards in a horizontal plane; the liquefied portion has a yellowish green fluorescence and is floored with whitish deposit.

On agar-agar.—No growth occurs at 37° C.

Agar-agar streak cultures at 20° C.—Growth is rapid, a greyish-white semi-translucent streak appearing in 24 hours about 2 m.m. wide and with irregular edges. Fluorescence is first perceptible on the second day when the streak is 3 m.m. wide. In eight days the agar-agar exhibits a brilliant greenish-yellow fluorescence, the growth itself being whitish and semi-opaque, and from 4 to 5 m.m. in width, with irregular wavy edges. The brilliant fluorescence persists for several weeks without fading.

On potato.—Grows as a dirty brown layer, spreading from the line of inoculation.

In broth.—Grows rapidly, giving rise to dense turbidity. A thin film forms at the surface, and the top layer of the broth acquires in time a marked greenish fluorescence. The sediment eventually becomes of a pale brown colour.

In milk.—Growth is similar to that of *B. fluorescens stercoralis*, but slower. No coagulation is produced, but after four days the upper layer becomes slightly yellowish and less opaque, owing to gradual digestion.

Temperature.—No growth whatever at 37° C. Grows well at 20° C.

Rapidity of growth.—Grows rapidly, attaining its maximum in a week or ten days.

Spore formation.—Not observed.

Need of oxygen.—Growth exceedingly scanty away from the surface. Oxygen seems necessary for the production of the fluorescent pigment.

Gas production.—Not observed.

Relation to gelatine.—Liquefies at first rapidly, later with increasing slowness.

Pigment production.—Produces a greenish-yellow fluorescent pigment, apparently allied to that yielded by *B. pyocyaneus.* No pyocyanin can be extracted by chloroform from agar-agar or gelatine cultures.

Relations.—Closely allied to *B. pyocyaneus*, but differing from it in its inability to grow at 37° C.

Bacillus fluorescens stercoralis.

Source.—Found in Homerton sewage and Barking sewage.

Form and arrangement.—Motile rods, about twice as long as broad, but of variable length. The breadth varies from 0·5 to 0·7μ, and the length from 0·8 to 1·6μ; longer forms rarely occur.

Motility.—Actively motile.

Growth on gelatine.—Gelatine plates.—Tiny circular colonies are visible under the microscope in 19 hours. In 24 hours they are visible to the naked eye. In two days the surface colonies are liquefied and appear as uniformly turbid circles 2 to 3 m.m. in diameter, with a whitish flocculent deposit at bottom. The deeper colonies are white, spherical and still very minute. In three days the colonies have increased in diameter up to 10 m.m., and are fused in places, the plate being mostly liquefied with distinct greenish yellow fluorescence and a sour faecal smell. On the fourth day the plate is completely liquefied and the fluorescence brighter.

Gelatine stab cultures.—In 19 hours there is already liquefaction at the surface as a small flat hemisphere 2 m.m. in diameter, which in two days attains a diameter of 6 or 7 m.m., and in three days reaches the walls of the tube, though not to a greater depth than 3 m.m. Little growth occurs along the needle-track, growth being practically confined to the surface. The liquefaction advances always in a horizontal plane, no cone being formed even at the first. In five days it extends 6 or 7 m.m. down the gelatine, and in a week about 12 m.m.; after this it progresses more slowly, and in 17 days has only advanced 25 m.m. from the surface. Greenish fluorescence is marked in the liquefied portion by the fifth day, a thin pellicle forms on the surface, and there is a thick whitish deposit at the bottom of the liquefied portion.

On agar-agar.—No growth occurs at 37° C. At 20° C. growth is rapid.

Agar-agar streak cultures.—In 24 hours the streak is 2 m.m. broad, of a greyish white colour, gummy and semi-translucent in appearance, with a thick slightly irregular edge. In two days the streak is 3 m.m. in width and distinct fluorescence is perceptible, and in a week the agar-agar shows brilliant greenish-yellow fluorescence. The streak ultimately attains a width of 4 to 5 m.m. In cultures of three weeks old or more the fluorescence fades to a yellowish brown tint and the growth becomes more transparent.

In broth.—Growth is very similar to that of *B. fluorescens liquefaciens;* marked green fluorescence appears in the surface layer after the third day.

In milk.—The milk does not coagulate, but is gradually digested, the upper layer becoming yellowish and semi-transparent after two days; this change gradually spreads downwards, and in five days there is a loose flocculent deposit at the bottom of the tube, the upper layers being semi-transparent and of a slightly yellowish-green tint.

Temperature.—No growth in any medium at 37° C. Grows well at 20° C.

Rapidity of growth.—Grows rapidly, attaining its maximum of growth in a week or 10 days.

Spore formation.—Not observed.

Need of oxygen.—Growth almost absent away from the surface. The fluorescent pigment is produced only in contact with the air.

Gas production.—Not observed.

Reaction on gelatine.—Liquefies rapidly.

Pigment production.—Produces a greenish yellow fluorescent pigment, apparently identical with that yielded by the preceding species, *B. cloacae fluorescens*, but fading more quickly. Liquefied plates have an unpleasant sour, somewhat fæcal smell.

Relations.—Allied closely to the preceding species, but is of somewhat smaller size, and liquefies gelatine more rapidly. It is also closely allied to *B. fluorescens liquefaciens*, but differs from it in the mode and rate of liquefaction of gelatine, and in the greater brilliancy of the fluorescence it produces in agar-agar cultures, in which it resembles *B. pyocyaneus*. From the last-named species it differs in its refusal to grow at 37° C.

CONCLUSIONS.

If we now proceed to contrast the micro-organisms which we have found present in London sewage, as above described, with those found in the air of London sewers, and described in a previous report, certain striking differences become at once apparent. The most striking is the absence of moulds from sewage. Out of the many thousand colonies which arose in the numerous plates which were made, moulds occurred only seven times, and of those seven moulds one, and one only, was allied to the common species existing in sewer air. This result coincides with that obtained by Jordan in the sewage of Lawrence, Mass. "Moulds," he says, "are found rarely in the sewage and effluents." Further the same striking result was obtained by Mr. Dibdin in a series of experiments he made on the number of micro-organisms in Barking sewage. In sewer air on the contrary moulds were found to be a predominating feature, forming on an average no less than 64·33 per cent. of the total colonies. The proportion, however, varies according to season; in winter it may be as low as 13·33 per cent., while in the summer months it may rise as high as 82·6 per cent. of the total. It is right, however, to point out that in our sewage experiments, agar-agar, which is a less favourable medium than gelatine for the growth of moulds, was employed, whereas in the estimation of the organisms present in sewer air gelatine was used. This, however, does not materially affect our results since most moulds grow well on agar-agar at a temperature of 22° C. Jordan, and Dibdin, moreover employed gelatine plates only.

Bacillus coli communis was found, with one exception, in each sample of sewage we examined, and varied in numbers from 20,000 to 200,000 per cubic centimetre. In addition we isolated in every case many bacilli which, although indistinguishable in cultural features from bacillus coli communis, nevertheless gave only one or at most two of the three characteristic chemical reactions. These bacilli in most instances far outnumbered the true bacillus coli communis. Nevertheless, neither B. coli communis nor any of its close allies were ever found in sewer air. Furthermore, we have obtained enormous numbers of sarcina, amounting in one case to over 300,000 per cubic centimetre, yet we have not found one single colony of Sarcina lutea which is so common in sewer air and fresh air.

The flora of sewage naturally embraces a very large number of different species of bacteria, but when we consider the enormous number of micro-organisms per cubic centimetre and the extreme degree of dilution that it is necessary to employ in making cultivations, it will be evident that we have probably isolated only those species which are present in comparatively large numbers. The following organisms are those which we have found to be present in numbers varying from 200,000 to 2,500,000 per cubic centimetre—bacillus fluorescens stercoralis, bacillus albus putidus, bacillus fluorescens liquefaciens, bacillus cloacae fluorescens, bacillus mycoides, proteus cloacinus, proteus Zenkeri, a streptococcus coagulating milk, staphylococcus pyogenes citreus, sarcina flava and its allies, and diplococcus albicans tardissimus. Other bacilli, which rapidly liquefy gelatine and produce a green fluorescence, were found in numbers varying from 10,000 to 200,000 per cubic centimetre.

A further difference between the bacteria of sewer air and those of sewage lies in the relative proportions of micrococci and bacilli. The bacteria of sewer air were found to consist mainly of micrococci, bacilli forming but a small proportion of the total species found. The bacilli found were B. subtilis, B. aureus, B. arborescens, B. helvolus, B. nigrescens, and B. acidi lactici. In sewage itself, however, there can be no doubt that bacilli preponderate over micrococci, probably in actual numbers, certainly in the numbers of species present. The large majority of those species which we have been obliged to pass over were bacilli. This was less noticeable in the fresh sewage taken from St. Bartholomew's Hospital, where the large number of streptococcus colonies materially altered the

ratio between the bacilli and micrococci; in the sewage taken from Snow-hill and Barking the bacilli showed a distinct numerical preponderance. This result again is in harmony with that obtained by other observers, indeed the results we have obtained show a somewhat smaller preponderance of bacilli over cocci than has been recorded by others. Jordan states that the comparative absence of micrococci from sewage is a striking and highly remarkable circumstance. In any case the facts again come out in striking confirmation of the view that the organisms present in sewer air are not derived to any essential extent from the sewage, or they would show to some degree the same relative ratio of micrococci to bacilli. The argument receives also important confirmation from other considerations. A large proportion of the bacteria of sewage have the property of very rapidly liquefying the nutrient gelatine used as a cultural medium, so large a proportion indeed as to make gelatine an impossible medium to employ in estimating their numbers. In sewer air, on the contrary, organisms rapidly liquefying gelatine were found to be practically absent, a fact impossible to explain on the assumption that sewer air is able to take up bacteria from sewage.

Again the number of micro-organisms existing in sewer air appears to be entirely dependent upon the number of micro-organisms existing in the fresh air at the same time and in the same vicinity. With the advance of the colder weather, and consequent rapid decrease in the number of micro-organisms in fresh air we find a corresponding decrease in the number of the micro-organisms of sewer air, although the temperature of the sewer air and sewage suffer but a comparatively slight variation.

If the organisms existing in sewer air were derived from those existing in sewage, then the flora of sewer air should bear a very close resemblance to the flora of sewage. When, however, we compare the organisms which have hitherto been isolated from sewer air with those species which we have found to be *predominant* in sewage, it is at once evident that they bear no resemblance whatever to one another, indeed we may go even further and state that so far as we are aware not a single colony of any of those species which we have found predominant in sewage has been isolated from sewer air. We consider therefore that the study of the sewage bacteria, on which we have been engaged, fully confirms the conclusion previously arrived at from the study of the micro-organisms of sewer air, viz., that there is no relationship between the organisms of sewer air and sewage.

It is possible that some of the ill effects which have been erroneously ascribed to sewer air may be due to subsoil air derived from soil polluted by constant infiltration of excremental matter through a leaky drain. It is a well recognised fact that subsoil air does at times gain access to our dwellings, either through the pressure of the wind on the surface of the ground or from currents induced by wide differences between the exterior and interior temperatures. Under such conditions it is possible that sewage may gradually extend through a permeable soil until its outer margin becomes sufficiently dry to give off micro-organisms to the subsoil air. Whatever the danger arising from this cause may be, it would in all probability be strictly limited in its effect.

PART II.

TYPHOID FEVER IN ITS RELATION TO SEWAGE.

The sources of the micro-organisms in London sewage are clearly diverse and manifold. It must contain species derived from the water of the different companies which supply the metropolis; these are numerous and varied, and so far they have not been sufficiently studied. It must contain species derived from the air, species again of which our knowledge is very limited. Further the superficial layers of the soil must add their quota of species brought down by surface water drainage, and the amount of organic matter in the soil of an old city must render that number a large one. Lastly the various putrescible organic matters which constitute the essential part of sewage will add a vast number of bacteria to the fluid; human excreta for example teem with organisms as do the waste and household refuse passing down into the sewers from houses and manufactories.

The ultimate bacterial flora of sewage is only in part determined by the organisms which pass into that fluid with its various constituents. Probably only a small proportion of these ultimately survive. For some species sewage forms a favourable nutrient medium, others appear speedily to perish in it. Those again which may be just able to grow in it under favourable circumstances, will be liable to be crowded out in the struggle for existence by their more vigorous competitors. The changes moreover induced in the sewage by the growth of certain bacteria, changes mostly of a fermentative and putrefactive character, will of necessity lead to alterations in its capacity for serving as a nutrient medium for some species; as its chemical composition alters some species may be favoured, others arrested in their growth. The struggle for existence, the survival of the fittest, will necessarily occur in miniature amongst these lowly organisms in sewage as elsewhere in the organic world. And thus the flora of sewage at the Barking and Crossness outfalls comes to be, as we have found it, very different from that of the perfectly fresh fluid as originally delivered into the drains.

It becomes, therefore, a matter of very great importance to determine the fate of certain pathogenic organisms in sewage. Amongst the diseases which have been attributed to sewer air, and to contamination of drinking water by sewage, two have especially attracted attention—cholera and typhoid fever, and the spread of diphtheria is held by some to be due to the same cause. During the period of our observations cholera was absent from London; Koch's comma bacillus could not have been expected to be present, and though all colonies resembling it were closely scrutinised, it was not found. The spread of diphtheria by sewage is at least a matter of doubt, and though sewer air has been held responsible for certain outbreaks of the disease, it seems clear that but a small part in the dissemination of the disease can be played by such a cause. The diphtheria bacillus can be easily recognised and its pathogenic power tested; yet though a careful look-out was kept for this organism we never found it. Typhoid fever stands on a different footing; there is no question that the specific poison of the disease, believed on very good

grounds to be the *Bacillus typhosus* of Eberth and Gaffky, passes from the body with the fæces, and that the excreta of typhoid patients constitute the main channel of infection in this disease. The disinfection of such excreta is a matter of extreme difficulty, and is as a rule very imperfectly carried out even in our fever hospitals. It hence results that sewage becomes a very important agent in the dissemination of the disease, and contamination of water supplies by sewage, the chief cause of typhoid epidemics. Our attention in the inquiry which we have carried out was therefore specially directed to the possible occurrence of the bacillus typhosus in London sewage, and every colony which seemed likely to belong to this species was the subject of careful investigation. It has been already mentioned that Bacillus coli communis, a normal inhabitant of the digestive tract of man and animals, and one of the commonest and most constant organisms present in sewage, bears a strong resemblance to the typhoid bacillus, and is in morphological and cultural characters closely allied to it. It can, however, be distinguished from it readily enough by certain chemical tests, and for the right understanding of the methods we have employed in searching for it, it is needful to go into this matter in some detail. In cultural characters, *i.e.* mode of growth on different nutrient media, the two organisms resemble one another so closely that even the most experienced bacteriologists would hesitate to diagnose one from the other by such means alone. In morphological characters they are better to be distinguished; the colon bacillus is shorter, and, owing to the possession of fewer flagella, is much less actively motile. Reliance is, however, chiefly to be placed in their different chemical reactions. The colon bacillus, when inoculated into sterilized milk and incubated at 37° C. produces coagulation of the milk in 24 to 36 hours; the typhoid bacillus has no such power, the milk remaining fluid for an indefinite period. The colon bacillus, when inoculated into nutrient gelatine, which is then liquefied at 25° C. and shaken so as to diffuse the bacillus throughout the mass of the nutrient medium, and then allowed again to solidify and incubated at 22° C., produces abundant gas bubbles as it grows, the typhoid bacillus under similar conditions produces no gas. The colon bacillus when grown in alkaline beef, broth, or in peptone solution, at 37° C. produces a body, probably indol, recognised by the development of a deep red colour on the addition of nitrous acid; the typhoid bacillus has no such power. These three tests form then an easy means of distinguishing the two organisms, and it may be affirmed that any given organism which in cultural characters resembles the bacillus typhosus, which is actively motile and grows into threads longer than those seen in B. coli communis, and which fails to produce coagulation in milk to form gas bubbles in gelatine shake preparations, and to give rise to indol in broth or peptone cultures, is at least indistinguishable from the typhoid bacillus, and may with every degree of probability be regarded as such. Further than this it is impossible to go; the lower animals do not, so far as we know, suffer from typhoid fever in the sense in which we recognise it in man, and hence inoculation experiments on animals do not help us to prove a given organism to be the actual virus of typhoid fever.

With these considerations clearly in view, we proceeded from the outset of our investigations to scrutinize carefully every colony which in any way seemed to resemble the typhoid bacillus. And in every case we failed to recognise it. We then became oppressed by a sense of mathematical improbability. The average amount of sewage produced in London amounts to 200 million gallons per diem. During June, 1894, 177 cases of typhoid fever were notified in London, to which may be added 13 cases of continued fever. Assuming each case to last a month, it may be supposed that during the early summer months, when typhoid is by no means prevalent, two hundred cases of the disease would represent a fair average of the number existing in London at any one time. A large number of these cases suffer from constipation and hence contribute very little fæcal matter to the general mass of sewage. Those who suffer from diarrhœa do so, as a rule, only for a certain portion of the time they are ill. Moreover, it must be remembered that in all outbreaks of typhoid a large number of cases of diarrhœa occurs, which, though not presenting the typical features of the disease, are probably truly typhoid in nature, and are capable of disseminating the infection. Any estimate, therefore, of the average amount of sewage contributed daily by the cases of typhoid in London must be purely conjectural, but a reasonable estimate of such average amount appears to us to be $\frac{1}{250\,000}$ part of the whole, and it must not be forgotten that every endeavour is made to disinfect this. The mathematical chances of detecting the typhoid bacillus in ordinary London sewage are therefore extremely remote. If the above assumption be correct, and if all the typhoid sewage be further assumed to be intimately mixed with the ordinary sewage, there would only be on an average one typhoid bacillus in every $\frac{1}{10}$ c.c. of sewage at the outfalls. But the largest amount of undiluted sewage with which we have found it possible to work is $\frac{1}{5000}$ of a c.c., and this only when 90 per cent. of the organisms present were inhibited by the addition of 0·05 per cent. carbolic acid, and incubation at 37° C.

Discouraged by these considerations we determined to search for the typhoid bacillus in sewers where it might be expected to be present in larger proportion. To this end we considered the different fever hospitals, and selected the Eastern Hospital at Homerton as likely to serve our purpose, since it has new drains easily accessible at the different manholes and inspection chambers, and since also the typhoid fever cases are located in a separate block of buildings. Having obtained the consent of the Metropolitan Asylums Board, we entered into correspondence with the resident medical superintendent, who kindly lent us all the aid in his power. It was agreed that no disinfection of the typhoid stools should take place for two days preceding our visit, and this was carried out. On the morning of May 23rd we visited the hospital at Homerton. There were at the time some 40 cases of typhoid in the block set apart for that disease, many being acute cases suffering from diarrhœa. The drain from the typhoid fever block joins that from the scarlet fever block, and is accessible by a manhole before it leaves the hospital. At this manhole we collected the sewage in a sterilized bottle, taking small samples at short intervals, so as to get a fair average specimen. It was unfortunately a wet day, so that the sewage was considerably diluted, and it contained also a large quantity of soapsuds. We returned immediately to the laboratory and at once proceeded to make cultivations from

the sewage. Knowing that the typhoid bacillus grows well at 37° C. and that it can grow in the presence of ·05 per cent. of carbolic acid, we made six agar-agar plates, with 1 c.c. of a dilution of 1 in 5,000 ; and after the addition of ·05 per cent. of carbolic acid to each plate they were incubated at 37° C. In this way, as we have shown earlier in this report, some 90 per cent. of the organisms present in sewage are prevented from growing, and so the task of searching for one particular organism capable of growing under these conditions is proportionately lightened. Bacillus coli communis and other allies of the typhoid bacillus can, however, also grow in such plates, and it was from these that we now had to differentiate any possible typhoid colonies. The chemical tests which have been mentioned above as distinguishing B. coli communis from B. typhosus suggested the following mode of procedure. From every colony which resembled the typhoid bacillus a sub-culture was at once made in sterilized milk and incubated at 37°. Those milk tubes which coagulated were rejected as being certainly not typhoid. From the ones which did not coagulate, streak cultures were made on gelatine ; some of these liquefied and were hence rejected as certainly not typhoid. Those which did not liquefy were each tested by shake cultures in gelatine, as to their power of gas production, and by cultures in broth to which the indol test with nitrous acid was applied. Some gave rise to both gas formation and the indol reaction, many others to one of the two reactions ; all such could be rejected, and at the end we were left with two colonies only which resembled B. typhosus in their morphological and cultural characters, which failed to produce coagulation in milk and which gave rise to no gas bubbles in gelatine and produced no indol in broth. These two colonies we hence regard as colonies of the typhoid bacillus, or at least as absolutely indistinguishable from that organism. Inasmuch as animals are not susceptible to typhoid fever in the strict sense of the term (*i.e.* since the typical lesions of human typhoid have not so far been reproduced experimentally in animals), it was useless to attempt any further verification of our results by inoculation experiments on animals, or we should have done so. We must be content to have shown that in the drain from the typhoid block of a fever hospital, when the stools have not been disinfected for two days a bacillus can be found which, so far as demonstration can go, is identical with that believed to be the actual cause of typhoid fever. So far as we are aware this important fact has never previously been demonstrated.

A week or two later we examined in the same way sewage taken from a drain in Bridge-street, Homerton, about a quarter of a mile from the hospital, which received the hospital sewage as well as that from the surrounding district. Exactly the same method of procedure was adopted, and the results proved entirely negative; no single colony could be referred to B. typhosus. This may have been due in part to the great dilution of the hospital sewage with that from the surrounding district, or in part to the fact that the typhoid stools were then being disinfected as usual.

EXPERIMENTS ON THE VITALITY OF THE TYPHOID BACILLUS IN SEWAGE.

We have shown above that the number of typhoid bacilli which gain access to our sewers, when compared with non-pathogenic microbes, is relatively very small, inasmuch as the amount of typhoid excreta forms but a minute proportion of the whole, and much even of this is in a measure disinfected. The same is true of the diphtheria bacillus and, during the prevalence of cholera, of the cholera vibrio. It is hence evident that it is a matter of the greatest importance to determine the duration of vitality of these pathogenic organisms under conditions which may be considered normal to their dissemination. The points to be determined are (1) whether normal sewage is a favourable or unfavourable soil for their life and growth, and (2) what influence, if any, the various non-pathogenic organisms present in such overwhelming numbers exert upon their pathogenic companions. It will be evident that the question is one of much complexity, demanding for its solution much detailed and laborious experiment. In order to gain some definite knowledge on these points, we have carried out a series of preliminary experiments with the bacillus of typhoid fever, and the results, though far from complete, appear to us of sufficient value to merit record.

The duration of vitality of the typhoid bacillus in sewage.—It was necessary in the first place to prepare sterile sewage in which to sow the typhoid bacilli. In the earlier experiments we did this by boiling sewage for a short period on two or three consecutive days, after it had undergone a preliminary filtration through ordinary filter paper. It became clear to us, however, that this was not a good method, since the process must of necessity affect the composition of the sewage and so render it abnormal. Not only will volatile organic matters be driven off by boiling, but the carbonates of lime and magnesia will be thrown out of solution together with certain soluble organic matters. We, therefore, adopted a method suggested to us by Dr. Klein, and employed the Berkefeld filter, which has sufficiently fine pores to prevent the passage of micro-organisms through it. The sewage was twice filtered through the Berkefeld filter and then heated to 60° C. in a sterile flask for 20 minutes on two consecutive days. In this way complete sterility was obtained, with the smallest possible departure from the normal as regards chemical composition. The sewage employed was from Barking or Crossness.

We first compared the typhoid bacillus with the colon bacillus as to their respective powers of vitality and multiplication in sewage which had been sterilised by heat.

Experiment I.—Two tubes of sterilised sewage were inoculated with *B. typhosus* and two with *B. coli communis*, and incubated at 37° C. After the lapse of 24 hours the tubes inoculated with *B. coli communis* showed abundant growth, while those inoculated with the typhoid bacillus showed no growth at all. After four days' incubation one typhoid tube showed very slight development, the other none, even when tested by sub-culture on gelatine.

Experiment II.—Three tubes of sewage sterilised by heat were inoculated respectively with a culture of *B. typhosus* obtained from the spleen of a fatal case of typhoid fever, with a culture of *B. coli communis* derived from the human intestine, and with one of *B. coli communis* obtained from the fresh sewage of St. Bartholomew's Hospital. All three cultures had been previously tested and gave typical reactions. The sewage tubes were incubated at 37° C. After two days the two B. coli tubes

were found to have undergone considerable growth, the fluid being milky in appearance. The typhoid tube remained clear. A platinum loop was dipped into each tube and cultures made on nutrient gelatine; the two B. coli tubes yielded very numerous colonies, the typhoid tube yielded only one colony after a long interval.

From the three sewage tubes, after the lapse of two days, three fresh sewage tubes were inoculated and incubated at 37° C. The typhoid tube was sterile; the B. coli tubes grew well, and from them third and subsequently fourth generations in sterile sewage were carried on. The fourth generation, three weeks after the commencement of the experiment, yielded very numerous colonies of *B. coli communis* when tested by inoculation on gelatine. It was also ascertained that after growing for four days in sewage, the second generation of *B. coli communis* still retained its powers of coagulating milk, of forming gas bubbles on gelatine, and producing indol in broth.

It thus appears that the harmless colon bacillus is able to grow and multiply abundantly in sewage sterilised by heat, when incubated at 37° C, and that it may be so cultivated for several generations; the typhoid bacillus, on the contrary, is unable to grow, and quickly perishes under these conditions. This constitutes a new and important distinction between these allied organisms.

It remained to test more accurately the duration of life of the typhoid bacillus in sterilised sewage, kept at 37° C.

Experiment III.—A tube of sewage sterilised by heat was inoculated with three drops of a broth culture of the typhoid bacillus (four days old). The cultivation was tested at intervals by removing a platinum loopful and spreading it over the surface of a gelatine tube. A sub-culture thus made immediately after its inoculation with the typhoid bacillus yielded more than 100 typhoid colonies. After the lapse of $21\frac{3}{4}$ hours a sub-culture yielded 15 colonies. After 50 hours only one colony was obtained, and after $73\frac{3}{4}$ hours none. The typhoid bacillus thus appears to die speedily when cultivated in sewage sterilised by heat and incubated at 37° C. death occurring within a few days.

A temperature of 37° C. is not, however, a normal condition in sewage, and we next attempted to determine the duration of vitality of the typhoid bacillus in sewage kept at the ordinary temperature of the air—20° C. The two following experiments show that at this temperature life is maintained for a longer period, though probably not beyond a fortnight.

Experiment IV.—A tube of sewage sterilised by heat was inoculated with the typhoid bacillus as in the preceding experiment, and incubated at 20° C. It was tested in the same way by sub-cultures on gelatine at suitable intervals, a control sub-culture being made at the outset, immediately after inoculation. After 24 hours a slight increase in the number of colonies was noticed, as if slight multiplication had occurred. The diminution in the number of colonies was thenceforward steady, till at the end of ten days a large capillary pipetteful of the sewage yielded only 30 or 40 colonies, and in 13 days none at all were found.

Experiment V.—A tube of sewage sterilised by heat was inoculated with one drop of a fresh broth culture of *B. typhosus*, and incubated at 20° C. A small quantity was removed on a platinum loop and transferred to gelatine immediately after inoculation to determine the number of colonies originally present, and the process was repeated at stated intervals. The results were as follows:—

Immediately after inoculation...	...	...	225	colonies	arose
After 24 hours	...	...	...	250	,,	,,
,, 68 ,,	...	...	...	140	,,	,,
,, 5 days	...	...	...	48	,,	,,
,, 7 ,,	...	...	...	13	,,	,,
,, 13 ,,	...	...	...	0	,,	,,

It is to be observed that the removal of so small a quantity of fluid as can be conveyed on a platinum loop is no fair test of the total absence of living bacilli from a culture, and it is quite possible that the removal of larger quantities might have shown living bacilli to be present after 13 days. Similar, or approximately similar, amounts would nevertheless be removed by a platinum loop on each occasion, so that the experiment clearly shows that although an attempt at growth may occur during the first 24 hours, the typhoid bacillus gradually but surely dies out in sewage, and is quite incapable of any active growth therein.

Sewage, therefore, even in the absence of the normal micro-organisms which it contains is clearly an unfavourable medium for the growth of the typhoid germ, whereas the colon bacillus can grow and multiply freely in it. It might be anticipated that in competition with other organisms able to grow well in sewage, the typhoid bacillus would die out even more speedily. The problem is a complex one, and in order to gain some idea of the influence which various non-pathogenic bacteria normally present in sewage would exert upon the life and growth of the typhoid bacillus when growing side by side with it, we selected certain of the organisms which we had frequently found during the progress of our observations, and experimentally tested their effect on the duration of vitality of the typhoid bacillus. In order to simplify the problem we confined our attention to those organisms which are incapable of growing at a temperature of 37° C., by which means it became easy to separate out the typhoid bacilli from any other organism or organisms to which it had been added, and to determine the number still alive after any given interval. When the agar-agar plate cultivations were incubated at 37° C., only the typhoid bacilli could grow. The organisms we selected were all capable of growing freely in sterile sewage at the ordinary temperature of the air, and were as follows—*Bacillus fluorescens liquefaciens*, *bacillus albus putidus*, and two of the species which we have described above as *bacillus fluorescens stercoralis* and *bacillus cloacæ fluorescens*. In the first two experiments the sewage was sterilised by boiling.

Experiment VI.—The typhoid bacillus grown in sterile sewage with *bacillus fluorescens stercoralis* and *B. albus putidus* respectively.

To two tubes each containing 10 c.c. of sterilised sewage, three drops of a fresh typhoid broth culture were added. To one of these tubes three drops of a fresh culture of *B. fluorescens stercoralis*, and

to the other a similar amount of a fresh culture of *B. albus putidus* were further added. The contents of each tube were well mixed, and they were allowed to remain at the temperature of the room screened from the action of direct sunlight. At the outset of the experiment, and at given intervals, the number of typhoid bacilli still living was determined, by making agar-agar plate cultivations with so much of the sewage as was removed on the end of a platinum loop, and the plates were incubated at 37° C.

The following results were obtained—

	Typhoid + B. fluorescens stercoralis.	Typhoid + B. albus putidus.
No. of colonies in original mixture ...	630	590
After 24 hours	148	570
After 76 hours	0	156
After 4 days	—	141
After 5 days	—	134
After 11 days	—	24

Experiment VII.—This experiment was in every detail similar to the preceding, but was carried out with *B. fluorescens liquefaciens*, *bacillus cloacæ fluorescens* and again with *bacillus fluorescens stercoralis*, three drops of fresh cultures of each respectively being added to the sterile sewage at the same time that it was inoculated with the typhoid bacillus.

The results were as follows—

	Typhoid + B. fluorescens stercoralis.	Typhoid + B. fluor. liquefaciens.	Typhoid + B. cloacæ fluorescens.
No. of colonies in original mixture ...	1,030	1,769	935
After 24 hours	1	949	946
After 3 days	0	1,010	235
After 4 days	—	20	—
After 7 days	—	0	—
After 10 days	(·5 c.c.) 135		18

It will be seen from these experiments that *B. fluorescens stercoralis* and *B. fluorescens liquefaciens* both exert a marked influence upon the duration of vitality of the typhoid bacillus in sewage, particularly the former. In no instance is there evidence of any increase in the number of typhoid bacilli. As no typhoid colonies arose after the third day in the case of *B. fluorescens stercoralis*, and after the 7th day in the case of *B. fluorescens liquefaciens*, it was desirable to know whether the typhoid bacilli had been completely destroyed or whether they had merely been so reduced in number as to escape detection when only a minute quantity of the mixture was used for making the plate cultivations. On making a plate culture with half a cubic centimetre of the mixture 135 colonies of the typhoid bacillus were obtained even after 10 days' exposure to the influence of *B. fluorescens liquefaciens*. The above figures must, therefore, be regarded as affording merely an indication of the rate at which the typhoid bacilli perished, and not as fixing definitely the date of their final destruction.

We determined to test this matter on a more extended scale, and in a more accurate manner. Therefore in the following experiment several slight modifications and improvements were introduced. The sewage was sterilised by filtration instead of by boiling. Larger quantities of sewage were employed for cultivation, and much greater precision was aimed at by measuring accurately the quantities of typhoid and other cultures used, and by employing for each agar-agar plate a carefully measured amount of the mixtures.

Experiment VIII.—Six small wide mouthed flasks, each containing $2\frac{1}{2}$ ounces of sterile sewage, were inoculated each with 0·5 c.c. of the same broth culture of the typhoid bacillus. Four of these flasks then received 0·2 c.c. respectively of fresh broth cultures of the following organisms:—*B. fluorescens stercoralis*, *B. fluorescens liquefaciens*, *B. albus putidus* and *B. cloacæ fluorescens*. A fifth flask received 0·2 c.c. of cultures of all four organisms—0·8 c.c. in all. The sixth flask, which was used as the standard, received only the typhoid bacilli. The flasks were agitated so as intimately to mix their contents and then kept in the dark at the temperature of the room. The number of typhoid bacilli per cubic centimetre was determined in each flask, from time to time, by abstracting a measured quantity of the fluid (from $\frac{1}{10}$ c.c. to 1 c.c.) and diluting it with sterilised distilled water to a given volume, and then making agar-agar plate cultures with 1 c.c. of the diluted material. The degree of dilution was diminished *pari passu* with the decrease in the number of the typhoid bacilli present, until it was possible to take relatively large quantities of the undiluted mixtures. The agar-agar plates were incubated at 37° C. As all the six flasks were inoculated with an exactly similar quantity of the same typhoid broth culture which had previously been thoroughly mixed, the number of bacilli per cubic centimetre in the flask containing typhoid bacilli alone was regarded as representing the average for the whole series. The results which we obtained are expressed in the following table—the number of bacilli per c.c. being calculated from the actual numbers observed.

Time of experiment.	Typhoid alone No. of colonies per c.c.	Typhoid+B. fluorescens stercoralis.	Typhoid+B. fluorescens liquefaciens.	Typhoid+B. albus putidus.	Typhoid+B. cloacæ fluorescens.	Typhoid+ Mixture of all four species.
At starting	1,048,000	—	—	—	—	—
1 day after	1,838,000	—	230,000	—	—	—
3 days „	506,000	107,000	80,000	531,000	839,000	228,000
5 „ „	3,000	800*	45,200	340,000	825,000	41,800
7 „ „	—	616*	26,400	3,000	78,000	27,800
10 „ „	0	502*	0	0	0	0

* 0·5 c.c. of the undiluted mixture employed for each plate.

Certain points calling for comment may be added to the above table. After the tenth day several of the flasks became contaminated with other organisms, so that exact estimation of the number of typhoid bacilli was impossible. We had reason, however, for believing that the total extinction of the typhoid bacilli did not take place quite as soon as the above figures indicate, since on the twelfth day cultivations from most of the flasks, made by taking 0·5 c.c. of the undiluted mixtures, showed a large number of colonies, many of which were certainly typhoid. The flask containing typhoid with *B. fluorescens stercoralis* proved perfectly sterile on the twelfth day, even though 0·5 c.c. of the mixture was used. The negative results on the tenth day from the other five flasks were obtained from plates made with the diluted mixtures, and it is possible that the distilled water used for dilution exercised an unfavourable influence on bacilli previously weakened by their sojourn in an unsuitable medium. In one flask, that containing typhoid with the mixture of all four organisms, some typhoid bacilli were found living even after the lapse of 30 days, when a sufficiently large amount of the undiluted mixture was used for cultivation.

In spite of possible errors thus arising during the later stages of this experiment, we believe that the figures in the table are reliable up to the end of the tenth day, at least no labour was spared to make them so. So far as deductions can be safely drawn from a single series of experiments, the following conclusions seem to be warranted. *Bacillus typhosus* seems capable of slight multiplication in sterilised sewage during the first 24 hours only, thenceforward it becomes gradually extinct. The presence of certain non-pathogenic organisms, commonly present in sewage, appears to affect its extinction. Of the four organisms which we tested *B. fluorescens stercoralis* alone seems to have any marked effect upon the vitality of *B. typhosus*, and this effect is practically absent when other organisms are present at the same time. The mixture of the four non-pathogenic bacteria had no effect in hastening the extinction of *B. typhosus*, indeed the reverse appears to have been the case.

These preliminary experiments are necessarily very incomplete, and afford only an indication of the probable fate of typhoid bacilli which gain access in a living condition to sewage. It seems however clear that sewage does not form a medium in which much, if any, growth is possible for them under natural conditions, and their death is probably only a matter of a few days or at most one or two weeks. But this degree of resistance may, nevertheless, be sufficient to allow of their being carried in sewage to remote distances and of their being able to produce disastrous results should they gain access to any water supply. As our knowledge accumulates, it becomes more and more evident that water supply and, as an incidental result, our milk supply constitute the chief channels of infection by which typhoid fever is communicated, and this is true also of cholera and possibly of other infectious diseases. It is, therefore, of the first importance to determine in an exhaustive manner how far sewage is a possible soil for the growth of these and other disease germs which admittedly gain access to it, and also to determine what precise influence their non-pathogenic companions may exert on them.

In the conclusions to Part I. of this report we endeavoured to show that sewer air has no power of taking up bacteria from the sewage with which it is in contact. A strong argument in favour of this view is the fact that the very organisms which are most abundant in sewage are precisely those which are absent from sewer air. In the course of previous experiments on sewer air, the nature of the organisms in some 1,200 litres of sewer air was carefully determined. Not once was *Bacillus coli communis*, or any of the predominant organisms of sewage found, though we have shown above that the former is present in sewage in numbers varying from 20,000 to 200,000 per cubic centimetre. If this be so, how infinitely improbable becomes the existence of the typhoid bacillus in the air of our sewers. That sewage is a common medium for the dissemination of typhoid is certain; that sewage-polluted soil may give up germs to subsoil air is possible; but that the air of sewers themselves should play any part in the conveyance of typhoid fever appears to us, as the results of our investigations, in the highest degree unlikely.

J. Parry Laws.
Frederick W. Andrewes.

13th December, 1894.

London County Council.

REPORT

ON THE

RESULT OF INVESTIGATIONS

ON THE

MICRO-ORGANISMS OF SEWAGE.

PART I.

Their relation to those of Sewer Air.

PART II.

Observations on the Bacillus of Typhoid Fever and its relation to Sewage.

BY

J. PARRY LAWS, F.I.C., and F. W. ANDREWES, M.B., M.R.C.P., D.P.H.

(*Printed by order of the Main Drainage Committee, 13th December*, 1894.)

No. 216. **Price** 4d. *Sold by Edward Stanford, 26 and 27, Cockspur-street, Charing-cross, S.W.*

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